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Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (*ompB*)

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To confirm the phylogenetic analysis previously inferred by comparison of the citrate synthase and rOmpA gene sequences (*gltA* and *ompA*, respectively), the rOmpB gene (*ompB*) of 24 strains of the genus *Rickettsia* was amplified and sequenced. rOmpB is an outer-membrane protein of high molecular mass, the presence of which can be demonstrated in most rickettsiae by immunological cross-reactivity in Western blots. No PCR amplification was obtained with *Rickettsia bellii* or *Rickettsia canadensis*. For the other rickettsiae, phylogenetic analysis was inferred from the comparison of both the gene and derived protein sequences by using parsimony, maximum-likelihood and neighbour-joining methods which gave the same organization. All nodes were well supported (>86% bootstrap values), except in the cluster including *Rickettsia africae* strain S and *Rickettsia parkeri*, and this analysis confirmed the previously established phylogeny obtained from combining results from *gltA* and *ompA*. Based on phylogenetic data, the current classification of the genus *Rickettsia* is inappropriate, specifically its division into two groups, typhus and spotted fever. Integration of phenotypic, genotypic and phylogenetic data will contribute to the definition of a polyphasic taxonomy as has been done for other bacterial genera.

Keywords: phylogenetic analysis, outer-membrane protein rOmpB, *Rickettsia*, taxonomy

INTRODUCTION

Rickettsia are obligate intracellular, Gram-negative bacteria, the vectors and reservoirs of which are mainly arthropods, although they may also infect vertebrate hosts. For many years the genus *Rickettsia* has been subdivided into three subgroups. The scrub typhus group included only one species, *Rickettsia tsutsugamushi*, which has recently been excluded from the genus *Rickettsia* on the basis of 16S rRNA gene sequence studies and reclassified in a new genus, *Orientia* (Tamura *et al*., 1995). The typhus group (TG) included three species: *Rickettsia typhi*, *Rickettsia prowazekii* and *Rickettsia canadensis*, and the spotted fever group (SFG) contained the other rickettsiae (Weiss & Moulder, 1984). Comparison of the sequences of the citrate synthase gene (*gltA*) and 16S rDNA demonstrated that *R. canadensis* should not be included in the TG or SFG (Roux & Raoult, 1995; Roux *et al*., 1997; Stothard & Fuerst, 1995). Currently, only *R. typhi*, the agent of murine typhus, and *R. prowazekii*, the agent of epidemic typhus, are included in the TG. Another species, '*Rickettsia felis*' was first suspected to cluster with *R. typhi* and *R. prowazekii* on the basis of immunofluorescence assays which indicated the close affinity of '*R. felis*' with members of the TG. Similarities between '*R. felis*' and TG rickettsiae were also present in the growth patterns of the organisms in cell culture and in immunoblotting studies (Azad *et al*., 1992; Radulovic *et al*., 1995). Subsequently, the classification of '*R. felis*' in the TG has been questioned based on results obtained from comparative studies of the sequences of 16S rDNA and *gltA* genes and the gene encoding the 17 kDa protein (Schriefer *et al*., 1994; Higgins *et al*., 1996).

The heterogeneous SFG contains rickettsiae which are generally transmitted by ixodid ticks. Recently, bac-

Abbreviations: SFG, spotted fever group; TG, typhus group.

The GenBank accession numbers for the *ompB* gene sequences of the *Rickettsia* strains used in this study are shown in Fig. 1.

Table 1. Rickettsial strains studied

Taxon	Strain	Source	Geographic origin	Human disease
Rickettsia conorii	Seven^T (Malish^T), ATCC VR-613^T	Unknown	South Africa	Mediterranean spotted fever
Rickettsia conorii (Indian tick typhus rickettsia)	ATCC VR-597	*Rhipicephalus sanguineus*	India	Mediterranean spotted fever
Astrakhan fever rickettsia	A-167	*Rhipicephalus pumilio*	Astrakhan region (formerly USSR)	Astrakhan fever
Israeli tick typhus rickettsia	ISTT CDC1	Human	Israel	Israeli spotted fever
Rickettsia sibirica	246^T, ATCC VR-151^T	*Dermacentor nuttali*	Former USSR	Siberian tick typhus
'*Rickettsia mongolotimonae*'	Marseille 1	Human	France	Unnamed spotted fever
Rickettsia parkeri	Maculatum 20	*Amblyomma maculatum*	Mississippi, USA	
Rickettsia africae	ESF-5	*Amblyomma variegatum*	Shulu Province, Ethiopia	African tick bite fever
Strain S	S^T	*Rhipicephalus sanguineus*	Armenia (formerly USSR)	
Rickettsia slovaca	13-B	*Dermacentor marginatus*	Slovakia	Unnamed spotted fever
Rickettsia honei (Thai tick typhus rickettsia)	TT-118^T, ATCC VR-599^T	*Ixodes* sp. or *Rhipicephalus* sp.	Thailand	Flinders Island spotted fever
Rickettsia honei	RB	Human	Tasmania	Flinders Island spotted fever
Rickettsia rickettsii	R^T (Bitterroot^T), ATCC VR-891^T	*Dermacentor andersoni*	Montana, USA	Rocky Mountain spotted fever
Rickettsia japonica	YM	Human	Japan	Oriental spotted fever
Rickettsia massiliae	Mtu1^T	*Rhipicephalus turanicus*	France	
Bar 29	Bar 29^T	*Rhipicephalus sanguineus*	Spain	
Rickettsia rhipicephali	3-7-6	*Rhipicephalus sanguineus*	Mississippi, USA	
Rickettsia aeschlimannii	MC16^T	*Hyaloma marginatum*	Morocco	
Rickettsia montanensis	M/5-6	*Microtus* sp.	Montana, USA	
Rickettsia australis	Phillips	Human	Australia	Queensland tick typhus
Rickettsia akari	MK^T (Kaplan^T), ATCC VR-148^T	Human	New York, USA	Rickettsial pox
Rickettsia helvetica	C9P9	*Ixodes ricinus*	Switzerland	
Rickettsia prowazekii	Breinl^T, ATCC VR-142^T	Human	Poland	Epidemic typhus
Rickettsia typhi	Wilmington^T, ATCC VR-144^T	Human	North Carolina, USA	Murine typhus

teria transmitted by insects have been proposed to belong to this group. These include the AB bacterium (Werren *et al.*, 1994) which has been found to cluster with *R. canadensis* (Roux & Raoult, 1995; Roux *et al.*, 1997). Also they include the pea aphid rickettsia (Chen *et al.*, 1996) and the PTB bacterium which clusters with *Rickettsia bellii* (Davis *et al.*, 1998). Currently, more than 20 serotypes of the rickettsiae are described in the SFG. The description of several new potential SFG rickettsiae each year has highlighted the necessity for reliable tools to infer phylogenetic analysis to enable bacteria to be classified within this group. Interest in the rOmpB protein resulted from its outer-membrane location and the presence of epitopes in this protein which are common to both SFG and TG rickettsiae as demonstrated by immunological cross-reactivity in Western blotting analyses and other serological investigations. The rOmpB gene (*ompB*) was first described in *R. prowazekii* and was found to be 4776 bp (Carl *et al.*, 1990). The gene encoding the rOmpB protein of *Rickettsia rickettsii* was then sequenced (Gilmore *et al.*, 1989, 1991). In *R. prowazekii* this crystalline layer protein represents 10–15% of the total protein mass (Ching *et al.*, 1996) and it was identified as the immunodominant species of surface protein antigen for most of the rickettsiae. Moreover, mAbs directed against rOmpB passively protected mice against lethal challenge of rickettsiae (Anacker *et al.*, 1987).

To confirm the phylogenetic analysis that we recently inferred by combining data obtained from the citrate synthase gene (*gltA*) and rOmpA gene (*ompA*) (Roux *et al.*, 1997; Fournier *et al.*, 1998), we describe in this report the DNA sequences of *ompB* of 24 representatives of the genus *Rickettsia*.

METHODS

Rickettsial strains. The strains used in this study are listed in Table 1.

Rickettsial cultivation. SFG and TG rickettsiae were grown on Vero cell monolayers as described previously (Roux *et al.*, 1997). When the degree of infection was optimal, as estimated by Gimenez staining, rickettsiae were harvested and cultures were centrifuged at 12000 ***g*** for 10 min, resuspended in medium and stored at −70 °C until nucleic acid purification was performed.

Nucleic acid purification and PCR amplification. Genomic DNA was extracted using Qiagen columns (QIAamp Tissue Kit), according to the manufacturer's instructions. PCR amplification reactions were performed using the oligonucleotide primers listed in Table 2. Most of the primers were designed following identification of suitable hybridization sites within conserved regions of the gene after alignment of the sequence of *R. rickettsii* (GenBank accession no. X16353) and *R. prowazekii* (M37647). Some of the primers were chosen specifically, after determination of a part of the sequence of the *ompB* gene, for *Rickettsia akari*, *Rickettsia australis* and *Rickettsia helvetica*. Each PCR was carried out in a Peltier model PTC-200 thermal cycler (MJ Research). Five microlitres of the DNA preparation was amplified in a 50 µl reaction mixture containing 25 pmol each primer, 200 µM (each) dATP, dCTP, dGTP and dTTP (Gibco), 1 U Elongase (Gibco); 2 µl buffer A and 8 µl buffer B. The following conditions were used for amplification: initial denaturation for 3 min at 95 °C was followed by 40 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 50 °C and extension for 1 min 30 s at 68 °C. The amplification was completed by holding the reaction mixture for 7 min at 68 °C to allow complete extension of the PCR products. Primer pairs used for all the bacteria were 120-M59 and 120-807, 120-607 and 120-1497, 120-1378 and 120-2399, 120-2113 and 120-2988, 120-2788 and 120-3599, 120-3462 and 120-4346, and 120-4232 and 120-4879. The seven

Table 2. Oligonucleotide primers used for PCR amplification and sequencing of rickettsial species

Fragment	Primer	Nucleotide sequence	rOmpB gene positions relative to the ORF	Organisms used
I	120-M59	CCGCAGGGTTGGTAACTGC	M59–M41	All
	120-807	CCTTTTAGATTACCGCCTAA	807–788	All except *R. helvetica*
II	120-607	AATATCGGTGACGGTCAAGG	607–626	All except *R. helvetica*
	120-1497	CCTATATCGCCGGTAATT	1497–1480	All except *R. helvetica*
III	120-1378	TAAACTTGCTGACGGTACAG	1378–1397	All except *R. helvetica*
	120-2399	CTTGTTTGTTTAATGTTACGGT	2399–2378	All except *R. helvetica*
IV	120-2113	CGATGCTAACGTAGGTTCTT	2113–2132	All except *R. akari* and *R. australis*
	120AA-2235	GGTACAGTTGGTACGCCTGC	2235–2254	*R. akari*, *R. australis*
	120-2988	CCGGCTATACCGCCTGTAGT	2988–2969	All except *R. akari* and *R. australis*
	120AA-3042	AATCTGTCCCGACTACACCA	3042–3023	*R. akari*, *R. australis*
V	120-2788	AAACAATAATCAAGGTACTGT	2788–2808	All
	120-3599	TACTTCCGGTTACAGCAAAGT	3599–3579	All
VI	120-3462	CCACAGGAACTACAACCATT	3462–3481	All except *R. akari* and *R. australis*
	120AA-3509	TACAGGAACTACAACCATTA	3509–3528	*R. akari*, *R. australis*
	120-4346	CGAAGAAGTAACGCTGACTT	4346–4327	All
VII	120-4232	GGTTTCTCATTCTCTCTATATGG	4232–4254	All except *R. helvetica*
	120-4879	TTAGAAGTTTACACGGACTTTT	4879–4857	All
	120-4489*	GTCTTCTGACGAAAACTACAA	4489–4509	All except *R. helvetica*
	120H-4108	TCGGTGCTGCTGTCGGTATCA	4108–4127	*R. helvetica*
	120H-4442*	GTCTTCTGACGAAAACTATAA	4442–4462	*R. helvetica*
I, II, III	120H-472*	CCTTTACTTGCAGCAGTACC	472–453	*R. helvetica*
R. helvetica	120H-558*	GGAAATGCACAGGCTACTTT	558–577	*R. helvetica*
	120H-1010*	CGGTGCTGACGGTAATAGTT	1010–1029	*R. helvetica*
	120H-1192*	GCACCGATATTACCGTTAAT	1192–1173	*R. helvetica*
	120H-1627*	CCGATANGCAAGGTATAATTG	1627–1647	*R. helvetica*
	120H-1746*	GACCAAGAGCCACATTTTGA	1746–1727	*R. helvetica*
	120H-2203	GCGTTACCTAAGAAGTAAGCC	2203–2183	*R. helvetica*

* Oligonucleotide primer used only for sequencing.

fragments were amplified for all the rickettsiae except *R. akari*, *R. australis* and *R. helvetica*. For *R. akari* and *R. australis*, a new pair of primers was chosen to amplify fragment IV: 120AA-2235 and 120AA-3042. To obtain amplification of fragment VI we used primers 120AA-3509 and 120-4346. For *R. helvetica*, it was possible to amplify fragments IV, V and VI. By determining the sequence of fragment VI and choosing a new primer, 120H-4108, which was used with primer 120-4879, we were able to amplify the 3′ end of the gene of *R. helvetica*. We determined a part of the 3′ end sequence of fragment IV and chose a new primer, 120H-2203, which, with 120-M59, enabled the amplification of a fragment of 2261 bp.

Sequencing reaction. PCR products were purified using a QIAquick Spin PCR Purification Kit (Qiagen) as described by the manufacturer. Sequencing reactions were carried out using a DNA sequencing kit (dRhodamine Terminator Cycle Sequencing Ready Reaction Fit with AmpliTaq Polymerase FS; Applied Biosystems) as described by the manufacturer. Sequence products were purified and resolved on 5% polyacrylamide gels (Long Ranger Singel packs, Type 377-36cm WTR; Tebu) and electrophoresis was performed with the ABI PRISM 377 DNA sequencer (Perkin Elmer). The same primers as those used for PCR amplification were used for the sequencing reactions. Primers 120H-4442 and 120-4489 were used to confirm the sequence of the 3′ end of the gene of *R. helvetica* and other rickettsiae. The primers 120H-472, 120H-558, 120H-1746, 120H-1010, 120H-1192 and 120H-1627 were chosen to obtain complete *R. helvetica* sequences of both DNA strands of the fragment amplified using the primer pair 120-M59 and 120H-2203.

Data analysis. The *ompB* sequences were translated into protein sequences using PC/GENE software (IntelliGenetics). The *ompB* sequences and rOmpB amino acid sequences were aligned using the multisequence alignment program CLUSTAL within the BISANCE environment (Dessen *et al.*, 1990). The percentage similarities were determined using the PC/GENE software package. The phylogenetic relationships between the studied representatives of the genus *Rickettsia* were

determined using the PHYLIP software package version 3.4 (Felsenstein, 1989). Distance matrices generated by DNADIST and PROTDIST were determined under the assumptions of Kimura (1980). These matrices were used to elaborate dendrograms using the neighbour-joining method (Saitou & Nei, 1987). The data were also examined by using parsimony and maximum-likelihood methods (DNAPARS, PROTPARS and DNAML in PHYLIP). A bootstrap analysis was performed to investigate the stability of the trees obtained. Bootstrap values were obtained for a consensus tree based on 100 randomly generated trees by using SEQBOOT and CONSENSE in the same package.

RESULTS

PCR amplification and sequencing

ompB fragments were amplified from all the studied strains of *Rickettsia* except *R. bellii* strain 369L42-1 and *R. canadensis* strain 2678.

The sequence of both DNA strands was determined twice. The *ompB* gene was sequenced between bases 1 and 5210 with respect to the *R. rickettsii* published sequence. Sequence similarity of 100% was obtained with the published sequences of *R. rickettsii* (Gilmore *et al*., 1989) and *R. typhi* (Hahn *et al*., 1993) but only 99·8% similarity was obtained with the sequence of *R. prowazekii* published by Carl *et al*. (1990). Different sequences were obtained for two strains of *Rickettsia conorii* (ATCC VR-597 and Seven^T) and two strains of *Rickettsia honei* (TT-118^T and RB). The percentage similarity between strains ATCC VR-597 and Seven^T was 99·8% for the DNA sequence and 99·5% for the protein sequence. For the TT-118^T and RB strains the values were 99·9 and 99·8% for the DNA and protein sequences, respectively. Between two different species the highest percentage similarity was obtained for *Rickettsia sibirica* and '*Rickettsia mongolotimonae*' (99·6% DNA, 99·1% protein). The lowest similarity was observed between *R. typhi* and *R. helvetica* (70% DNA, 51·7% protein).

Phylogeny inference

The dendrograms obtained with the three different tree-building analysis methods used showed similar organization for most of the rickettsiae, whether inferred from the gene or the derived protein sequences. The two representatives of the TG, *R. prowazekii* and *R. typhi*, clustered together. We identified four monophyletic groups within representatives of the SFG: *R. akari* and *R. australis* clustered together and *R. helvetica* was alone on a separate branch. Another group consisted of *Rickettsia montanensis*, *Rickettsia aeschlimannii*, *Rickettsia rhipicephali*, *Rickettsia massiliae* and Bar 29. The fourth group could be divided into three subgroups. One included *R. honei* and *R. rickettsii*; the second included *Rickettsia africae*, strain S, *Rickettsia parkeri*, *R. sibirica* and '*R. mongolotimonae*'; and the third group consisted of the *R. conorii* complex (*R. conorii* strains, Astrakhan fever rickettsia and Israeli tick typhus rickettsia). *Rickettsia slovaca* and *Rickettsia japonica* did not cluster with any *Rickettsia* species. All the nodes were well supported ($\geqslant$ 87% bootstrap values for dendrograms inferred from comparison of gene and derived protein sequences; Fig. 1) except in the subgroup including *R. africae*, strain S and *R. parkeri* (from 40 to 46% in the dendrogram inferred from *ompB* sequences; from 54 to 62% in the dendrogram inferred from derived protein sequences).

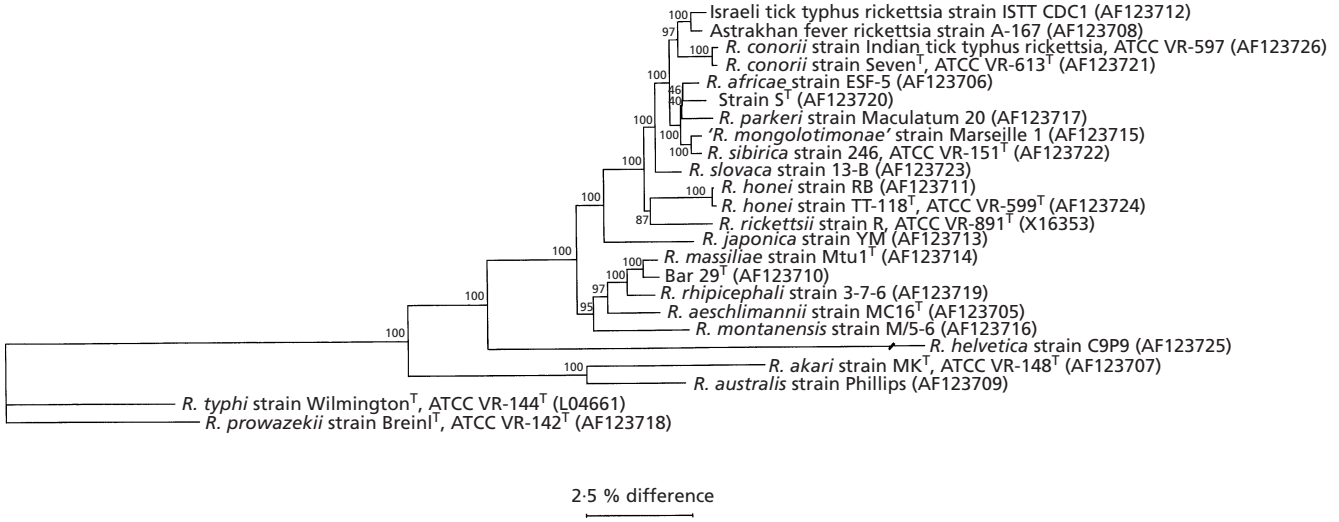

Fig. 1. Dendrogram representing phylogenetic relationships between *Rickettsia* species inferred from comparison of *ompB* gene sequences. The sequences were aligned using the multisequence alignment program CLUSTAL, which is a part of the BISANCE software package. Phylogenetic relationships were inferred with the PHYLIP software package version 3.4. The evolutionary distance values were determined by the method of Kimura and these values were used to construct a dendrogram by the neighbour-joining method. The numbers at nodes are the proportion of 100 bootstrap resamplings that support the topology shown. GenBank accession numbers for the sequences used are in parentheses.

DISCUSSION

For strict intracellular bacteria such as rickettsiae, few phenotypic characters are expressed or can be readily studied and the taxonomy of these organisms is based mainly on genomic criteria. Recently, we have demonstrated that the phylogenetic analysis of rickettsiae is possible by comparing their DNA sequences. Reliable analysis of all the isolates of the genus *Rickettsia*, as confirmed by high bootstrap values, was only obtained, however, by combining the data derived from the comparison of almost complete sequences of *gltA* and *ompA* (except tandem repeats) (Roux *et al*., 1995; Fournier *et al*., 1998). Since the *gltA* gene encodes citrate synthase, a highly conserved enzyme essential in the central metabolic pathways of the rickettsiae, the sequences we obtained for this gene were only significantly different between organisms distantly related to each other. Also, *ompA* is a gene encoding an outer-membrane protein specific to the SFG rickettsiae but not to all representatives of the genus *Rickettsia*. As it is important to compare the sequences of several genes to evaluate their use in establishing rickettsial phylogeny, as suggested by Olsen & Woese (1993), and since we were also looking for a versatile DNA target available for all the rickettsiae, we considered *ompB* to be a good candidate for further study.

rOmpB is a crystalline S-layer protein which has been characterized in nearly all phylogenetic lines (Sleytr & Messner, 1983), although the majority of sequenced S-layer genes have been found to have little sequence identity (Bahl *et al*., 1997). S-layers have been shown to be involved in the rigidity and shape of bacteria, to function as molecular sieves and ion traps and to be promoters for cell adhesion and surface recognition (Sleytr & Messner, 1988). In our experiments using PCR amplification with consensus primers established by comparison of published sequences of rickettsiae, we were able to determine the sequences of *ompB* in all the rickettsiae we studied except for *R. canadensis* and *R. bellii*. This result is consistent with that obtained previously using *gltA* and suggests that these two rickettsia, and also the AB bacterium, were the first to diverge from the common ancestor of this group (Roux *et al*., 1997). Methods such as 'genome walking' should be considered in future experiments to obtain the gene sequence of these rickettsiae as only one specific primer will be necessary (Siebert *et al*., 1995). The rapid rate of evolution we found for this gene in *R. helvetica* compared to that of the other representatives of the genus that we studied was surprising. Using PFGE, we have shown previously that the genome of *R. helvetica* (1400 kb) is longer than that of most of the other SFG rickettsiae (1200–1300 kb) (Roux & Raoult, 1993). Further studies are needed to determine if, during its evolution, *R. helvetica* was exposed to particular selective forces imposed by factors affecting its arthropod or mammalian hosts. Nevertheless, we can consider that its phylogenetic position between the *R. massiliae* subgroup and the cluster including *R. akari* and *R. australis* is established.

The phylogenetic position of *R. rickettsii* and *R. honei*, which grouped together, was well supported by analyses on *ompB*, contrary to analysis on *gltA* and *ompA*. The other subgroups, however, were identical to those identified by Fournier *et al*. (1998) and high bootstrap values were calculated for all supporting nodes. The single remaining problem is the organization inside the cluster including *R. parkeri*, strain S and *R. africae*. Bootstrap values for the supporting nodes were not significant, even though the clusters were the same as those determined by *ompA* sequence comparison. Differences were noted in sequences of isolates of the same species; within the species *R. conorii* differences were found between the Indian tick typhus strain (ATCC VR-597) isolated from a *Ripicephalus sanguineus* tick and strain Seven^T isolated from a patient with Mediterranean spotted fever; within the species *R. honei* differences were found between TT-118^T isolated from a tick in Thailand and the RB strain which is a human isolate from Flinders Island. These findings are the first to indicate differences between the above isolates as previously they were shown to be identical by analysis of their *gltA* and *ompA* sequences. Gilmore *et al*. (1991) also described heterogeneity in the DNA sequence of virulent and avirulent strains of *R. rickettsii*. Further investigations are indicated to confirm the heterogeneity between different isolates of the same species and try to understand the significance of the finding.

According to modern biosystematics, including phylogeny and population genetics, it is evident that the present classification and nomenclature used for the genus *Rickettsia* are inadequate. The division of the genus into only the TG and SFG is not appropriate as *R. canadensis* and *R. bellii* have to be excluded from the TG and the SFG, respectively. The significance of the SFG has to be reconsidered as the rickettsiae included in this group in fact form well defined, distinct clusters of organisms.

Currently, phylogenetic studies have shown that several groups can be determined within the genus *Rickettsia*. In one group are most of the rickettsia, mainly the *R. conorii* complex (*R. conorii*, Astrakhan fever rickettsia, Israeli tick typhus rickettsia), *R. sibirica*, '*R. mongolotimonae*', *R. africae*, strain S, *R. parkeri*, *R. slovaca*, *R. rickettsii*, *R. honei* and *R. japonica*. The second group includes *R. massiliae*, Bar 29, *R. rhipicephali*, *R. aeschlimannii* and *R. montanensis*. To date these bacteria have only been isolated from ticks and their pathogenicity in humans has yet to be determined. The third group includes *R. typhi* and *R. prowazekii*, the agents of endemic and epidemic typhus, respectively, and it appears appropriate then for this group to be named TG. The other groups are more difficult to define precisely. *R. helvetica* seems to have followed its own distinct evolutionary path. It has recently been recognized as a human pathogen (Nilsson *et al*., 1999). *R. akari* and *R.*

Table 3. G+C content, DNA–DNA similarity and antibiotic susceptibility for different representatives of the genus *Rickettsia*

Taxon	G+C (mol%)*	DNA–DNA similarity/ *R. rickettsii* (%)*	DNA–DNA similarity/ *R. prowazekii* (%)*	Erythromycin susceptibility†	Rifampin susceptibility†
R. rickettsii group:					
R. conorii	32–33	91–94	NT	R	S
R. rickettsii	32–33	NT	47	R	S
R. sibirica	32–33	70–74	NT	R	S
R. massiliae group:					
R. rhipicephali	32–33	NT	NT	R	R
R. montanensis	NT	73	NT	R	R
R. massiliae	NT	NT	NT	R	R
R. helvetica group:					
R. helvetica	NT	NT	NT	R	S
R. akari group:					
R. akari	32–33	46	NT	R	S
R. australis	NT	53	NT	R	S
R. prowazekii group:					
R. prowazekii	29–30	47	NT	S	S
R. typhi	29–30	42	72	S	S
R. canadensis group:					
R. canadensis	29–30	47	45	R	S
R. bellii	NT	NT	NT	R	S

* NT, Not tested.

† R, Resistant; S, sensitive.

australis are genetically closely related and should be included in their own group. '*R. felis*', which is also genetically closely related, could be the third species in this group. More data are required to determine if *R. bellii*, *R. canadensis* and rickettsiae recently described in insects (AB bacterium, Pea aphid rickettsia, PTB bacterium) should be included in the same group.

Bacterial taxonomy should reflect the classification and nomenclature of the different representatives and allow the identification of unknown organisms (Vandamme *et al.*, 1996). Expressed features such as morphology, physiology and ecology should also be reflected. Polyphasic taxonomy, which integrates data on numerous characteristics of organisms, seems to be the best approach for rickettsial taxonomy as has been shown to be the case with other bacterial genera (Vandamme *et al.*, 1996). The different groups we have defined by phylogenetic analysis may serve as a basis to establish taxonomy and may be confirmed by other criteria such as natural antibiotic susceptibility (Rolain *et al.*, 1998) which is usually supported by genetic specificity (Drancourt & Raoult, 1999), G+C content and DNA–DNA hybridization, but few data are currently available (Walker, 1989; Table 3). For other bacterial genera it has been determined that different strains belong to the same species if they share 70% or greater DNA–DNA relatedness (Wayne *et al.*, 1987). For rickettsiae, considering 70% as the threshold value, *R. rickettsii*, *R. conorii*, *R. sibirica* and *R. montanensis* would belong to the same species. So particular criteria would have to be determined for these bacteria as no rickettsiologist would accept this situation. A remaining problem is that rickettsiae are intracellular bacteria and, to date, the *in vitro* culture of some of the organisms has not been possible. The taxonomic position of these bacteria is now mainly based on gene sequence comparisons. Such phylogenetic analysis is only reliable, however, when it is based on sequences long enough to generate significant bootstrap values. Studies on limited gene sequence fragments could be useful for identifying bacteria, but not to reliably classify them. As primers are now available to amplify *gltA*, *ompA* and *ompB*, it will be judicious to establish the whole sequence of these genes to obtain a reliable phylogeny for all the bacteria identified as representatives of the genus *Rickettsia*.

Recently, the sequence of the entire genome of *Rickettsia prowazekii* was reported (Andersson *et al.*, 1998) and we have undertaken the sequencing of the whole genome of *R. conorii*. Comparison of the two genomes will give a direct insight into the genetic basis and molecular characteristics of each of these bacteria. Such new information will greatly improve our understanding of the taxonomy of the genus *Rickettsia*.

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